Great Ormond Street Hospital for Children NHS Foundation Trust

Chemical Pathology Services

External User Guide

Updated August 2012

New website: www.labs.gosh.nhs.uk/

Accredited Medical Laboratory

Reference No: 0250

INTRODUCTION

Chemical Pathology Laboratory, Great Ormond Street Hospital for Children NHS Foundation Trust (GOSH), is a CPA accredited laboratory providing a wide range of Chemical Pathology analyses with a special interest in the diagnosis and monitoring of inborn errors of metabolism.

The laboratory is fully staffed between 9 am and 5.30 pm Monday to Friday and staff will be available for any enquiries you may have. For sample requirements and general enquiries not dealt with by this guide or for results, please contact the helpline in the first instance.

For other enquiries, advice on investigations, clinical advice and interpretation or to request an urgent analysis, the duty biochemist is available on bleep 020 7405 9200 (hospital switchboard) bleep 0589. The on duty clinical staff member can also be contacted by a long range message pager, via the hospital switchboard, out of hours.
For additional information see website http://www.labs.gosh.nhs.uk/

This handbook contains of all the tests currently performed in house by the Department. There are a number of investigations that are available to GOSH Clinicians provided by External Referral Laboratories; details of which can be found in the Internal User's Guide (CCL002).

Sample reception
Chemical Pathology Reception Level 1
Paediatric Laboratory Medicine
Camelia Botnar Building
Great Ormond Street Hospital for Children
Great Ormond Street
London
WC1N 3JH

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DEPARTMENT SECTIONS AND PHONE NUMBERS

Departmental Office (**results enquires**)	020 7405 9200 ext 5076 020 7829 8624 (fax) Email: chempath.pa@nhs.net
Helpdesk (**general enquires**)	020 7405 9200 ext 5009

Metabolic Laboratory	020 7405 9200 ext 5225
Enzyme Laboratory	020 7405 9200 ext 2509/2440 Email: gos-tr.ENZYME@nhs.net
Specimen Reception/Routine Laboratory	020 7405 9200 ext 5009
Newborn Screening Laboratory	020 7829 8383 (DD) Email: gos-tr.enquiresgosnbs@nhs.net

REQUESTING

A request giving the following information must accompany the specimen (apart from newborn screening tests), a minimum of three identifiers are required:-

Patient ID:	surname or family name forename or personal name date of birth (many reference ranges are age dependent) sex (some reference ranges are sex related) patients reference i.e. Hospital number, laboratory, NHS number
Specimen:	type date and time of collection
Assay(s) required:	
Clinical details:	include medication, diet, fasting or fed sample
Sender:	name of sender address for report and invoice urgent contact, name, phone number (if different from sender):

Labeling of Specimens
Specimens should be legibly labeled with a minimum of three patient identifiers (see above) along with the date and time of collection, type of specimen and specimen reference. To avoid results being wrongly attributed to patients, unlabelled samples or samples that do not match the name on the request form cannot be processed by the laboratory.

SAMPLE COLLECTION/HANDLING

Requirements for sample collection and processing are listed under individual analyses further on in this booklet.

Abbreviations used:				
	Li hep	Lithium heparin	L	Liver
	Plain	Plain container	M	Muscle
	RBC	Erythrocytes	F	Fibroblasts
	WBC	Leucocytes	FB	Fetal blood
	S	Serum	VL	Vacuolated lymphocytes
	P	Li hep plasma	CV	Chorionic villus
	B	Whole blood	CCV	Cultured chorionic villus
	BS	Blood spot	AF	Amniotic fluid

STORAGE

Samples should be sent to us as soon as possible after collection. However, if storage is unavoidable, guidance for sample storage is given under individual test.

Requesting additional tests and sample retention

If the sample is still available and sufficient in volume and is viable, additional tests may be added by phoning the Helpdesk. On occasion, the requestor may be asked to send a further request form with details of the test required.

Samples are retained in accordance to the Guidelines published by the Royal College of Pathologists and the Institute of Biomedical Science. The retention and storage of pathological records and specimens (4th edition, 2009). All samples are stored for a minimum of 48 hours after the report has been issued; most samples are stored for at least two weeks and many are stored for longer periods. Please contact the Helpdesk for further advice.

PACKING

The packing requirements for samples are specified under each analyte further on in the booklet.

General and room temperature

All specimens must be in leakproof containers. Seal cap of container with 'parafilm' or similar waterproof tape. Wrap each container with sufficient absorbent material to completely absorb the contents in case of breakage. There should be no contact between containers. Place the container(s) and packing in plastic bag and seal the bag. Place the sealed bag, together with the request form, in a rigid fibre or plastic outer case. The outer case should be sealed with tape.

NOTE – the request form must **not** be inside the plastic bag with the specimen.

Ice

Pack specimens as above. Place the ice in a leak-proof container (use a plastic bottle or bag). Ice should not come into direct contact with the specimen container to avoid risk of contamination or labels becoming illegible. Place the ice and specimen(s) in a plastic outer container and seal with waterproof tape. Include sufficient ice to cover any possible delays in delivery.

Ice packs are suitable for **a journey time of less than 6 hours**. However, **DO NOT place ice packs from –20 °C freezer immediately next to whole blood or cells.**

Dry Ice [Solid CO_2]

Pack the specimens as above. The outer pack must be an insulator, e.g. expanded polystyrene. State "CONTAINS SOLID CO_2" on the outside. Seal outer case with tape. Include sufficient solid CO_2 to cover any possible delays in delivery.

TRANSPORT

First class post

When sending specimens by first class post, the packaging MUST comply with UN3733 packaging regulations and postal regulations.

The package must be labeled 'PATHOLOGICAL SPECIMEN' and may only be sent 1st class letter post. Where first class post is indicated this assumes that delivery will be made by the next day. Please DO NOT POST on Friday or before a UK Bank Holiday.

Courier or express delivery.
A reliable service should be used and instructed to take the specimens to the Reception in Chemical Pathology in the Camelia Botnar Building.

TURNAROUND TIME

Turnaround time given is the anticipated time taken between sample receipt and report under normal operating conditions. Where the assays are batched and performed infrequently, the time is given as a range up to the maximum anticipated time. Time taken for sample transport and posting the report should be added to this. **Where appropriate, abnormal results will be phoned or faxed to the sending laboratory**. In cases where results are required more urgently, please contact the department to discuss your requirements prior to sending specimens so that samples can be fast tracked.

NEWBORN SCREENING

Blood spot assays to screen for phenylketonuria (PKU), congenital hypothyroidism, medium chain acyl coA dehydrogenase deficiency, sickle cell disorders and cystic fibrosis in the neonatal period are available.

Sample requirement: 4 good blood spots collected between day 5 and 8 (day of birth = day 0) on a standard screening card, dried at room temperature, and enclosed in a glassine cover. Please provide the dates of birth and sampling. Send at room temperature by post immediately. Results will be available within 4 working days

FACTORS AFFECTING THE PERFORMANCE OF TESTS AND THEIR INTERPRETATION

METABOLIC INVESTIGATIONS

What samples?
It is important to check the fluid in which the metabolites of interest most obviously accumulate, e.g. urine for organic acids. The next part of this booklet indicates the sample type required for the investigations offered. When indicated (e.g. because of metabolite instability), it is necessary to make arrangements with the laboratory prior to collecting the sample.

When?
The time of the sample collection is crucial where characteristic metabolites accumulate only intermittently in the samples. Whenever possible, patients should be investigated during periods when they are unwell. Samples should be taken as soon as possible after admission, before changes in treatment and diet lead to the disappearance of relevant metabolites.

Sample integrity
Bacterial activity in poorly preserved samples produces a rise in pH and can lead to both the appearance of bacterial metabolites and the breakdown of important components, especially sugars and some amino acids. Samples with a high pH may not be analyzed for this reason. Faecal contamination of urine produces a similar effect. Dilute urine makes the detection of urinary constituents unreliable and samples with creatinine concentration >1 mmol/L are preferred.

Diet
Some metabolic disorders are related to a particular dietary intake or are produced only in the fasting state. Investigations should be carried out, as far as possible, on samples taken at the time the patient was symptomatic. Dietary restrictions or feeding may cause characteristic metabolites to disappear and result in false negative results. Dietary metabolites may interfere with organic acid, amino acid or carbohydrate chromatograms. Patients receiving intravenous amino acid mixture may have amino aciduria, amino acidaemia or organic aciduria. Information on the type of diet and the timing of the sample in relation to meals will aid in the interpretation of these complex analyses.

Drugs
Drugs influence metabolic investigations by analytical interference or by modifying metabolic processes. Details of all medication should be provided with metabolic investigations.

Exchange transfusions / blood transfusions
These may affect the analytes measured in blood and especially erythrocytes. When requesting tests in such patients, check whether adequate time has lapsed since the last transfusion. For assays of enzymes and metabolites in erythrocytes, the time interval should be 6 weeks.

Other factors include
Age of specimen
Time of specimen separation
Specimen storage
Specimen haemolysis, icterus and lipaemia
Fasting state

BIOCHEMICAL INVESTIGATION OF A SUDDEN INFANT DEATH

If an inborn error of metabolism is suspected in an infant who died suddenly, collect the following samples as soon as practicable to minimize post mortem changes; blood spots, bile spots, plasma, urine, CSF, aqueous humor. Blood stained CSF and urine should be spun and separated immediately and this should be recorded. Freeze at –20 °C. Skin biopsy can also be taken (see fibroblasts). Please discuss the request with the duty biochemist – 020 7405 9200 bleep 0589 before sending the sample.

METABOLIC INVESTIGATION IN A MORIBUND CHILD

The diagnosis of metabolic disease cannot be made after death unless the correct specimens have been appropriately collected. If metabolic disease is suspected and the child seems likely to die before a diagnosis can be made, it would be advisable to collect the following specimens:

Blood - 10 ml in a heparinised tube. Separate plasma promptly. Freeze the bulk of the plasma, the remaining plasma and red cells should be kept at 4 °C.

Urine - 20 ml in a plain container and deep freeze.
Blood spots for acylcarnitine

Bile spots for acylcarnitine

DNA - If the condition is one in which DNA studies are likely to be helpful, take 10ml blood into an EDTA tube and deep freeze the whole blood.

Tissue biopsies (liver, muscle, heart – Label the plain container with the type of tissues prior to taking the biopsies. Pre-cool a plain container in the deep freeze. Obtain dry ice, liquid nitrogen or a freezing pack. Make a small boat with a piece of aluminium foil and place it on the dry ice / freezing mixture. Take the biopsy (as many cores as possible, minimum two) and put it immediately in the boat, it should freeze immediately and thereafter should be not allowed to thaw at any time. Wrap up the core in the foil and put it in the pre-chilled container, making sure that the cap is tight and immediately replace in the deep freeze (-40 °C or lower). A small part should be put into glutaraldehyde and if necessary some into formalin, but the majority should be frozen for chemistry and enzymology.

Skin Biopsy - See fibroblasts

ASSAY DIRECTORY

Codes

Tests highlighted in turquoise are analysed in the Metabolic Laboratory

Test highlighted in green are analysed in the Enzyme Laboratory

Tests highlighted in yellow are analysed in the Routine Laboratory

AMINO ACID DISORDERS

Test	Sample requirement	Sample handling	Turnaround
Amino acids			
Plasma	0.5 ml li hep plasma	Separate ASAP. Freeze immediately. Send frozen	1 – 2 w
Urine	5 ml fresh random urine	Freeze immediately. Send frozen	3 – 6 w
CSF	0.2 ml clear CSF	Freeze immediately. Send frozen	1 – 2 w
Blood spot - branched chain	4 blood spots on std card	Send by first class post.	4 d
Homocysteine			
Plasma	0.2 ml li hep plasma	Separate ASAP. Freeze immediately. Send frozen	1 – 2 w
Urine	5 ml fresh random urine	Freeze immediately	3 – 6 w
Succinylacetone	5 ml fresh random urine	Freeze immediately. Send frozen	2 - 4 w
Sulphite	5 ml fresh random urine	Freeze immediately. Send frozen	72 h
Sulphocystine	5 ml fresh random urine	Freeze immediately. Send frozen	3 – 6 w

Methionine load for the diagnosis of homocystinuria

Preparation: Fast overnight (6 hours for infants).

Pre-load: At the beginning of the test, empty bladder and discard sample. Collect 1ml blood in a lithium heparin tube, separate and precipitate immediately.

Methionine load: Empty bladder. Give L-methionine (100 mg/kg body weight) orally over 5 minutes in a flavoured drink or as tablets. Collect 1 ml blood in lithium heparin tubes at 2, 4 and 6 hours. On each occasion, separate and precipitate immediately. Collect all urine passed over the 6 hours after giving the methionine load. Send plasma supernatant and urine on solid CO_2 for amino acid analysis.

Maple syrup urine disease enzyme diagnosis (Leucine decarboxylating system) (Skin fibroblasts)

Send a skin biopsy sample in the culture medium (See Appendix 1).

CARBOHYDRATE METABOLISM DISORDERS

Congenital Disorders of Glycosylation (CDG)

Initial investigation – serum transferrins isoelectric focusing at Institute of Neurology. If type I pattern CDG 1a & 1b should be excluded first. If patterns are abnormal but clinically indicated, then measure enzymes.

Test	Type	Sample handling		Turnaround
Phosphomannomutase	WBC	5-10 ml well mixed li hep whole blood with no clots	Send whole blood at ambient temp. to reach lab ideally within 24 h of collection	up to 6w
	F	skin biopsy into culture medium or saline	Send at ambient temp. by courier. Do not freeze	up to 10w
Phosphomannose isomerase	WBC	5-10 ml well mixed li hep Whole blood with no clots	Send whole blood at ambient temp. to reach lab ideally within 24 h of collection	up to 6w
	F	skin biopsy into culture Medium or saline	Send at ambient temp. by courier. Do not freeze	up to 10w

Galactose/ Fructose metabolism Disorders

Test	Type	Sample handling		Turnaround
Reducing substances Sugar chromatography	U	5 ml fresh random urine	Freeze immediately. Send frozen	3 – 6 w
Galactose -1-phosphate uridyl-transferase [Gal-1-PUT]	RBC	2 ml li hep whole blood. No transfusion prior 6 w	Send whole blood at ambient temp. to reach lab ideally within 48 h of collection	2 - 4 w
Galactokinase	RBC	2 ml li hep whole blood No transfusion prior 6w. ***Contact lab prior sampling***	Send whole blood at ambient temp. to reach lab within 24 h of collection	4–6 w
UDP-galactose	RBC	2 ml li hep whole blood No transfusion prior 6 w Contact lab prior sampling	Send whole blood at ambient temp. to reach lab ideally within 24 h of collection	4–6 w
Galactose-1 Phosphate	RBC	2 ml li hep whole blood	Send whole blood at ambient temp. to reach lab ideally within 24 h of collection	4–6 w
Fructose-1-P aldolase	L	liver biopsy	Freeze immediately. Send frozen without thawing	4-6 w

Glycogen Storage Disorders (GSD)

Test	Type	Sample handling		Turnaround
Ia Glucose-6-phosphate hydrolase	L	Fresh liver biopsy	Contact enzyme lab prior to sampling. Do not freeze.	4-6 w
Ib Glucose-6-phosphate translocase	L	Fresh liver biopsy	Contact enzyme lab prior to sampling. Do not freeze.	4-6 w
II α-1,4-glucosidase	BS,	Blood spots, 5 ml li	Send whole blood at	4-6 w

(acid maltase)	WBC	hep blood+ EDTA blood for vacuolated lymphocytes	ambient temp. to reach lab ideally within 24 h of collection	
III glycogen debrancher	WBC	5-10 ml li hep whole blood	Send whole blood at ambient temp. to reach lab ideally within 24 h of collection	4-6 w
	M, L	muscle / liver biopsy	Freeze immediately Send frozen	4-6 w
	F	skin biopsy into culture Medium or saline	Send at ambient temp. Do not freeze	4-6 w
IV glycogen brancher	WBC	5-10 ml li hep whole blood	Send whole blood at 4-6 w ambient temp. to reach lab within 18 h of collection	4-6 w
	M, L	muscle / liver biopsy	Freeze immediately Send frozen	4-6 w
	F	skin biopsy into culture Medium or saline	Send at ambient temp. Do not freeze	4-6 w
V phosphorylase	M	muscle biopsy	Freeze immediately Send frozen	4-6 w
VI phosphorylase	WBC	5-10 ml lip hep whole blood	Send whole blood at ambient temp. to reach lab ideally within 24 h of collection	4-6 w
	L	liver biopsy	Freeze immediately Send frozen	4-6 w
VII phospho fructokinase	M	muscle biopsy	Freeze immediately Send frozen	4-6 w
IX phosphorylase b Kinase	RBC	5 ml li hep whole blood	Send whole blood at ambient temp. to reach lab ideally within 24 h of collection	4-6 w
	L	liver biopsy	Freeze immediately Send frozen	4-6 w
IX fructose-1,6 bisphosphatase	WBC	5-10 ml lip hep whole blood	Send whole blood at ambient temp. to reach lab ideally within 24 h of collection	4-6 w
	L	liver biopsy	Freeze immediately Send frozen	4-6 w

Pompe CRIM testing *– contact the Enzyme Laboratory Tel:* 020 7405 9200 ext 2509/2440

Glycolytic enzymes

Test	Type	Sample handling		Turnaround
Phospho-glucomutase	WBC	5 ml li hep whole blood	Send whole blood at ambient temp. to reach lab ideally within 24 h of collection	4-6 w
	M, L	muscle / liver biopsy	Freeze immediately Send frozen	4-6 w

FATTY ACID OXIDATION DEFECT / HYPOGLYCAEMIA

Test	Type	Sample handling		Turnaround

3-(B)Hydroxy-butyrate (BOHB)	P	0.3 ml li hep plasma	Freeze immediately. Send frozen. Provide glucose result.	1 - 2 w
Free fatty acids (non-esterified fatty acids, NEFA)	P	0.3 ml li hep plasma	Freeze immediately. Send frozen. Provide glucose result.	1 - 2 w
Acetoacetate	B	perchloric acid supernatant	Freeze immediately (see appendix for protocol). Send frozen	1 – 3 w
Organic acids	U	5 ml fresh random urine	Freeze immediately. Send frozen	2 – 4 w
Acylcarnitines	BS	4 blood spot on standard card	Send by first class post	1 - 2 w

Diagnostic fast
All the above investigations to be carried out at the beginning and end of the fast under close medical supervision in a Hospital unit experienced in carrying out these tests (not advisable in patients under 18 months or under 5 kg in weight)

LACTATE / PYRUVATE DISORDERS

Test	Type	Sample handling		Turnaround
Lactate	P	2 ml fluoride oxalate plasma	Separate plasma assay ASAP	6 h
	B	perchloric acid precipitation (see appendix for protocol)	Freeze immediately Send frozen	1 – 2 w
	CSF	0.2 ml clear CSF	Freeze immediately Send frozen	1 – 2 w
Pyruvate	B	perchloric acid precipitation (see appendix for protocol)	Freeze immediately Send frozen	1 – 2 w
	CSF	perchloric acid precipitation (see appendix for protocol)	Freeze immediately Send frozen	1 – 2 w

LYSOSOMAL STORAGE DISORDERS (LSD)

Initial investigations/monitoring

Test	Type	Sample handling		Turnaround
Glycosaminoglycans	U	5 ml fresh random urine	Send at ambient temp. by special post	up to 4 w
Sialic acid	U	5 ml fresh random urine	Send at ambient temp. by special post	up to 4 w
Ceramide trihexoside (CTH) Globotriaosylceramide (GL3/GB3)	U	5 ml fresh random urine	Send at ambient temp. by special post	up to 4 – 6 w
Tetrasaccharides (hex4) Tetraglucose (glc4)	U	5 ml fresh random urine	Send at ambient temp. by special post	up to 4 – 6 w
Vacuolated lymphocytes	B	2 ml EDTA whole blood (see page 22)	Send at ambient temp. by special post (done in Histopathology)	**Contact histopathology**

Individual enzyme

Assays available individually for the diagnosis of lysosomal storage disorders are listed below with samples suitable for the assay. Turnaround is 4-6 weeks

Disease	Assay	Tissue
Mucopolysaccharidoses		
I-Hurler	α-iduronidase	WBC, F
II-Hunter	iduronate-sulphatase	WBC, P, F
IIIA-Sanfilippo A	heparan sulphamidase	WBC, F
IIIB-Sanfilippo B	α–N-acetyl-glucosaminidase	WBC, P, F
IIIC-Sanfilippo C	N-acetyltransferas	WBC, F
IIID-Sanfilippo D	N-acetyl-glucosamine-6-sulphatase	WBC, F
IVA-Morquio A	N-acetyl galactosamine-6-sulphatase	WBC, F
IVB-Morquio B	β-galactosidase	WBC, F
VI-Maroteaux-Lamy	arylsulphatase B	WBC, F
VII-Sly	β-glucuronidase	WBC, P, F
Multiple enzyme defects		
Mucolipidosis II (I-cell)	multiple hydrolases	P, VL, F
Mucolipidosis III (pseudo Hurler)	multiple hydrolases	P, VL, F
Multiple sulphatidosis	multiple sulphatases	WBC, P, F
Gangliosidoses		
G_{M1} gangliosidosis	β-galactosidase	WBC, VL, F
G_{M2} gangliosidoses:		
Tay Sachs / B1 variant	hexosaminidase A	WBC, P, F
Sandhoff	total β-hexosaminidase	WBC, P, F
Leucodystrophies		
Krabbe	galactocerebrosidase	WBC, F
Metachromatic	arylsulphatase A	WBC, F
Glycoproteinoses		
Fucosidosis	α-fucosidase	WBC, P, VL, F
α-Mannosidosis	α-mannosidase	WBC, P, VL, F
β-Mannosidosis	β-mannosidase	P, WBC, F, VL
Schindler	α-N-acetyl galactosaminidase	P, WBC, F
Sialidosis	α-neuraminidase	WBC, VL, F
Aspartylglucosaminuria	aspartylglucosaminidase	P, F
Galactosialidosis	α-neuraminidase/ β-galactosidase	WBC, VL, F
Other lipid storage disorders		
Fabry	α-galactosidase	WBC, P, F
Gaucher	β-glucosidase	WBC, F
	chitotriosidase	P
Niemann-Pick A & B	sphingomyelinase	WBC, VL, F,
Wolman & cholesteryl ester storage disease (CESD)	acid esterase	WBC, VL, F,
Neuronal ceroid lipofuscinoses (*Batten disease****)***		
Infantile (INCL, NCL1, CLN1)	palmitoyl protein thioesterase	WBC, F
Classic late infantile (LINCL, NCL2, CLN2)	tripeptidyl peptidase I	WBC, F
Transport defects		
Cystinosis	cystine	WBC, F
Sialic acid storage	sialic acid	U, VL, F

NB: **Prenatal diagnosis** is available for these disorders.

Grouped Enzyme Screens for Lysosomal Disorders

The lysosomal storage disorders can be grouped according to clinical features and a group of enzyme assays can be carried out on a single blood sample which provides both white blood cells and plasma for analysis. The clinical signs of a lysosomal storage disease may eventually develop to give a classic picture but diagnosis at an earlier stage can be more difficult, e.g. while Type II Gaucher disease leads to hepato/splenomegaly, neurological signs may be more obvious initially. To meet this and other concerns all patients have plasma chitotriosidase measured to exclude Gaucher disease and other LSDs. Palmitoyl protein thioesterase and tripeptidyl peptidase I which are deficient in infantile (INCL, NCL1, CLN1) and classic late infantile (LINCL, NCL2, CLN2) neuronal ceroid lipofuscinosis are assayed in all patients under 16 years with neurological problems, and also in adult patients if these disorders are suspected.

It is important that the laboratory is given full clinical details in order to carry out the appropriate combination of tests. Turnaround time is 6 - 8 weeks
Note: Some diseases may present under more than one heading.

Neurodegenerative screen
Evidence of neurological regression, hypotonia, fits, etc.

Disease	Enzyme
G_{M1} gangliosidosis	β-galactosidase
G_{M2} gangliosidoses:	
Tay Sachs / B1 variant	hexosaminidase A
Sandhoff	total β-hexosaminidase
Krabbe leucodystrophy	galactocerebrosidase
Metachromatic leucodystrophy	arylsulphatase A
Fucosidosis	α-fucosidase
α-Mannosidosis	α-mannosidase
β-Mannosidosis	β-mannosidase
Schindler	α-N-acetyl galactosaminidase
MPS VII-Sly	β-glucuronidase
I cell disease	I cell screen

Plasma chitotriosidase is assayed in all patients to exclude Gaucher disease

All patients under 16 years of age are tested for:

Infantile neuronal ceroid lipofuscinosis(INCL, NCL1, CLN1)	palmitoyl protein thioesterase
Classic late infantile neuronal ceroid lipofuscinosis (LINCL, NCL2, CLN2)	tripeptidyl peptidase I

Dysmorphic screen
The first line test for a dysmorphic child is screening for a mucopolysaccharidosis by urine GAGs. The following enzymes are indicated if a mucopolysaccharidosis is excluded.

Disease	Enzyme
G_{M1} gangliosidosis	β-galactosidase
Sialidosis	α-neuraminidase
Galactosialidosis	α-neuraminidase/ β-galactosidase
Fucosidosis	α-fucosidase
α-Mannosidosis	α-mannosidase
I cell disease	I cell screen

β-Mannosidosis	β-mannosidase
MPS VII-Sly	β-glucuronidase
Multiple sulphatidosis	arylsulphatase A
Aspartylglucosaminuria	aspartylglucosaminidase
Schindler	α-N-acetyl galactosaminidase

Plasma chitotriosidase is assayed in all patients to exclude Gaucher disease

Hepato/splenomegaly screen

For those patients with hepatomegaly and or splenomegaly suspected of having a lysosomal storage disorder.

Disease	Enzyme
G_{M1} gangliosidosis	β-galactosidase
Sialidosis	α-neuraminidase
Galactosialidosis	α-neuraminidase/ β-galactosidase
Gaucher	β-glucosidase
Niemann-Pick A & B	sphingomyelinase
Wolman & CESD	acid esterase
Fucosidosis	α-fucosidase
α-Mannosidosis	α-mannosidase
I cell disease	I cell screen
β-Mannosidosis	β-mannosidase
MPS VII-Sly	β-glucuronidase

In all patients with hepato/splenomegaly plasma chitotriosidase is assayed

Cherry red spot screen

For patients with a cherry red spot on the macula.

Disease	Enzyme
G_{M1} gangliosidosis	β-galactosidase
G_{M2} gangliosidoses:	
Tay Sachs / B1 variant	hexosaminidase A
Sandhoff	total β-hexosaminidase
Niemann-Pick A	sphingomyelinase
Sialidosis	α-neuraminidase
Galactosialidosis	α-neuraminidase/ β-galactosidase
Krabbe leucodystrophy	galactocerebrosidase

Angiokeratoma screen

For patients with an angiokeratoma.

Disease	Enzyme
Fabry	α-galactosidase
Fucosidosis	α-fucosidase
Sialidosis	α-neuraminidase
Galactosialidosis	α-neuraminidase/ β-galactosidase
Adult G_{M1} gangliosidosis	β-galactosidase
α-Mannosidosis	α-mannosidase
β-Mannosidosis	β-mannosidase
Schindler	α-N-acetyl galactosaminidase
Aspartylglucosaminuria	aspartylglucosaminidase

DNA Analysis

The Enzyme Laboratory works closely with the Clinical Molecular Genetics Laboratory at Great Ormond Street Hospital to offer mutational analysis for many of the lysosomal storage disorders. It is essential to test for the presence of the polyA mutation encoding a **pseudodeficiency** of arylsulphatase A in all patients with low arylsulphatase A activity. For other disorders the Enzyme Laboratory will advise if mutational analysis is available and/or appropriate when a diagnosis is made.

PRENATAL DIAGNOSIS

Prenatal diagnosis is available for the following disorders. It is important that the diagnosis in the index case has been confirmed in an appropriate tissue. The tissues suitable for assay are stated in the table. **It is <u>essential</u> to contact the Enzyme Laboratory (020 7405 9200 ext 2509) before taking any samples for prenatal diagnosis to discuss your requirements and transport arrangements.**

For chorionic villus specimens, it is our policy to assay the villi directly, where appropriate, and then to check equivocal results or confirm diagnosis of an unaffected fetus on cultured cells.

Direct and cultured cell assays are charged separately and an additional charge is made for the cell culture. For amniotic fluid samples where the assay is performed on cultured cells, the cost of the cell culture is charged additionally.

Lysosomal storage disorders

Mucopolysaccharidoses, mucolipidoses and multiple sulphatidosis

Following amniocentesis, electrophoresis of amniotic fluid glycosaminoglycans (GAGs) is carried out on all pregnancies at risk for a mucopolysaccharidosis, mucolipidoses II and III or a multiple sulphatidosis.

Disorder	Enzyme	Samples
Mucopolysaccharidoses		
I Hurler / Scheie	α-iduronidase	CV, CCV, CAC
II Hunter	iduronate sulphatase	CV, CCV, AF, CAC
IIIA-Sanfilippo A	heparan sulphamidase	CV, CCV, CAC
IIIB-Sanfilippo B	α-glucosaminidase	CV, CCV, CAC
IIIC-Sanfilippo C	N-acetyltransferase	CV, CCV, CAC
IVA-Morquio A	N-ac galactosamine-6-sulphatase	CV, CCV, CAC
IVB-Morquio B	β-galactosidase	CV, CCV, CAC
VI-Maroteaux-Lamy	arylsulphatase B	CV, CCV, CAC
VII-Sly	β-glucuronidase	CV, CCV, AF, CAC
Mucolipidosis II (I-cell)	multiple lysosomal hydrolases	CCV, AF, CAC
Mucolipidosis III (pseudo-Hurler)	multiple lysosomal hydrolases	CCV, AF, CAC
Multiple sulphatidosis	multiple sulphatases	CV, CCV, AF, CAC,
Lipidoses		
GM1 gangliosidosis	β-galactosidase	CV, CCV, CAC
GM2 gangliosidoses:		
Tay Sachs	hexosaminidase A	CV, CCV, CAC
Sandhoff	total β-hexosaminidase	CV, CCV, AF, CAC
Krabbe leucodystrophy	galactocerebrosidase	CV, CCV, CAC
Metachromatic leucodystrophy	arylsulphatase A	CV, CCV, CAC
Fucosidosis	α-fucosidase	CV, CCV, CAC
β-Mannosidosis	β-mannosidase	CV, CCV, CAC
α-Mannosidosis	α-mannosidase	CV, CCV, CAC
Schindler	α-N-acetyl galactosaminidase	CV, CCV, CAC
Sialidosis	neuraminidase	CV, CCV, CAC
Galactosialidosis	α-neuraminidase/ β-galactosidase	CV, CCV, CAC

Fabry	α-galactosidase	CV, CCV, AF, CAC
Gaucher	β-glucosidase	CV, CCV, CAC
Niemann-Pick A & B	sphingomyelinase	CV, CCV, CAC
Wolman & CESD	acid esterase	CV, CCV, CAC
Other lysosomal disorders		
Sialic acid storage	sialic acid	CV, CCV, AF, CAC
Cystinosis	cystine	CV, CCV, CAC
Pompe (GSD type II)	α-glucosidase	CV, CCV, CAC
Neuronal ceroid lipofuscinoses		
Infantile (INCL, NCL1, CLN1)	palmitoyl protein thioesterase	CV, CCV, CAC
Classic late infantile (LINCL, NCL2, CLN2)	tripeptidyl peptidase I	CCV, CAC
Glycogen storage disorders		
GSD II (Pompe)	α-glucosidase	CV, CCV, CAC
GSD IV	brancher	CV, CCV, CAC
Urea cycle disorders		
OCT deficiency	ornithine carbamoyl transferase	fetal liver
CPS deficiency	carbamoyl phosphate synthase	fetal liver
Arginase deficiency	arginase	FB
Argininosuccinate lyase deficiency	argininosuccinate lyase	FB
Other disorders		
Maple syrup urine disease	release of $^{14}CO_2$ from leucine	CV

ORGANIC ACID DISORDERS

Test	Type	Sample handling	Turnaround
Organic acids incl. methylmalonate	5 ml fresh random urine	Freeze immediately. Send frozen	2 – 4 w
N-acetylaspartate	5 ml fresh random urine	Freeze immediately. Send frozen	2 – 4 w
	2 ml amniotic fluid	By arrangement. Send by courier	as arranged
Biotinidase	0.2 ml li hep plasma	Freeze ASAP. Send frozen	1 – 3 w

PEROXISOMAL DISORDERS

Very long chain fatty acids, includes phytanate & pristanate	0.5 ml li hep plasma	Separate immediately. Send by 1st class post.	2 – 4 w

UREA CYCLE DISORDERS

Amino acids	P	0.5 ml li hep plasma	Separate ASAP. Freeze immediately. Send frozen	1 – 2 w
Organic acids, includes orotic acid	U	5 ml fresh random urine	Freeze immediately. Send frozen	2 – 4 w
N-acetylglutamate Synthase	Discuss with enzyme lab			up to 6 w
Arginase	RBC	5 ml li hep whole blood	Send whole blood at ambient temp. to reach lab ideally within 24 h of collection	up to 6 w
	L	liver biopsy	Freeze immediately. Send frozen	up to 6 w
Argininosuccinate Synthase	L	liver biopsy	Freeze immediately. Send frozen	up to 6 w
Argininosuccinate Lyase	RBC	5 ml li hep whole blood	Send whole blood at ambient temp. to reach lab ideally within 24 h of collection	up to 6 w
	L	liver biopsy	Freeze immediately. Send frozen	up to 6 w
Carbamoyl Phosphate synthase	L	liver biopsy	Freeze immediately. Send frozen	up to 6 w
Ornithine carbamoyl Transferase	L	liver biopsy	Freeze immediately. Send frozen	up to 6 w

OTHER INHERITED METABOLIC DISORDERS

Hypophosphatasia

Phospho-ethanolamine	U	5 ml fresh random urine	Freeze immediately. Send frozen	3 – 6 w

Disaccharidase Deficiencies

Enzymes	jejunum	2 mg jejunum biopsy	Snap freeze in liquid N2. Send frozen on solid dry ice. Also see appendix 1.	up to 8 w
Sugar chromatography	stool	walnut size stool	Freeze immediately. Send frozen	3 – 6 w

Glycerol kinase deficiency

Organic acids, includes glycerol	U	5 ml fresh random urine	Freeze immediately. Send frozen	2 – 4 w
Glycerol kinase	F	skin biopsy into culture medium or saline	Send at room temperature. Do not freeze	up to 10w

NEUROBLASTOMA SCREEN

Test	Type	Sample handling		Turnaround
HVA	U	5 ml fresh random urine	Freeze ASAP. Send frozen	3 d
VMA	U	5 ml fresh random urine	Freeze ASAP. Send frozen	3 d

OTHER TESTS

Antimullerian hormone (AMH)	P, S	0.5 ml EDTA plasma or serum	Separate and freeze plasma / serum immediately after collection. Send frozen	up to 2 m
Busulphan	P	1 ml EDTA blood at 0, 0.5, 1, 1.5, 2, 4, 7 h	Arrange with lab prior to sampling. Send sample on ice immediately to local Lab. Separate and freeze plasma ASAP Label samples clearly with time of collection.	Same day. Must be pre-booked.
Copper	P	0.4 ml li hep plasma	Separate plasma ASAP	1 – 2 w
	U	10 ml aliquot of 24 h	Collect 24h urine into acid washed container. Note 24 h volume.	2 – 4 w
Inhibin B	P, S	0.5 ml li hep plasma or serum	Separate and freeze plasma / serum immediately after collection. Send frozen	up to 3 m
Insulin	S, P	0.3 ml serum/plasma	Separate plasma. Send on ice.	1 d
Manganese	P	0.5 ml whole blood in Trace metal container	Send whole blood by first class post	2 – 4 w
Selenium	P	0.4 ml li hep plasma	Separate plasma ASAP Send by first class post	2 - 3 w
Sugar chromatography	U, F	5 ml random urine walnut size stool	Freeze immediately. Send frozen	3 – 6 w
Vitamin A	S	0.5 ml serum	Protect from light Separate serum ASAP Send by first post	2 - 4 w
Vitamin E	S	0.5 ml serum	Separate serum ASAP Send by first post	2 - 4 w
Zinc	P	0.4 ml li hep plasma	Separate plasma ASAP Send by first class post	1 – 2 w

ISOENZYMES

Alkaline phosphatase isoenzymes	P, S	0.5 ml li hep plasma / serum	Send by 1st class post	up to 4 w
Amylase isoenzymes	P, S	0.5 ml li hep plasma / serum	Send by 1st class post	up to 5 w
Creatine kinase isoenzymes	P, S	0.5 ml li hep plasma / serum	Separate and freeze plasma / serum immediately after	up to 4 w

			collection. Send frozen	

RENAL TUBULAR MARKERS

Retinol binding Protein [RBP]	U	1 ml fresh random urine	Freeze soon after collection	1 - 3 w
N-acetylglucosaminidase [NAG]	U	1 ml fresh random urine	Send by 1st class post	1 - 3 w

APPENDIX 1: Special Enzyme Assays

It is important that full clinical details (especially presence or absence of neurological features, hepatosplenomegaly, dysmorphic features) are given on the request form so that appropriate assays can be carried out. Please let us know if the mother is pregnant as we can advise on prenatal diagnosis.

Sample requirements
Enzyme assays are classified under separate diagnostic groups with abbreviations for samples where appropriate. These abbreviations are explained below.

WBC (leucocytes) for white cell enzymes
Unless specified, enzymes are assayed according to the clinical details given. Blood transfusion within 4 weeks may interfere with the result and sampling at this time is best avoided if possible.

Send 5 – 10 ml well mixed blood in lithium heparin (minimum of 5mls). The sample must not contain any clots, heparinise the syringe if the patient is difficult to bleed. Send the whole blood sample to reach the laboratory ideally within 24 hours of sample collection (the shorter the interval, the better the quality of the sample). For most enzymes up to 48 hours is acceptable. However WBC cystine it is **essential** that the **sample is received within 24 hours**. Send by courier or Royal Mail Special Next Day delivery to arrive before 14:30 on a normal working day. Please avoid sending samples on a Friday in case of delays in transport.

The turnaround time for these assays is approximately 6 weeks.

RBC (erythrocytes)
Blood transfusion in the previous 6 weeks invalidates results.

Send 2 ml heparinised blood to arrive in the laboratory within 24 hours of sample collection, **except for galactokinase + epimerase which has to be assayed on the day of sample collection and should be arranged with the enzyme laboratory at least a day in advance.** Send by courier or Royal Mail Special Next Day delivery.

P (plasma) I cell screen etc
Send 1 ml plasma from a lithium heparin blood sample, to reach the laboratory within 24 hours of collection. Send by courier or Royal Mail Special Next Day delivery.

F (fibroblasts) from skin biopsies
Taking a skin biopsy:

Proceed under aseptic conditions. Have sterile culture medium ready. The forearm and axilla are suitable sites. Swab the skin with alcohol or chlorhexidine (not iodine or betadine). Approximately 0.2 ml to 0.4 ml of 0.5% lignocaine or similar local anaesthetic is injected intradermally and just subcutaneously. Take a 3 mm punch biopsy (full thickness skin) or ellipse 4 mm x 2 mm, immediately transfer the skin to the culture medium. IN EXCEPTIONAL circumstances, sterile dextrose / saline may be used. Keep at 4 °C or room temperature (DO NOT FREEZE) and send by courier or datapost. Fill the container to the top to avoid any airlock.

Storage: 4-8 ^{o}C for 24 hours in sterile saline, 3 to 5 days in sterile culture medium. It will take up to 6 weeks to grow fibroblasts.

VL (vacuolated lymphocytes)
Send 3 unstained and 1 stained blood film made and stained by your Haematology department or 200 μl blood in EDTA. This test is performed in the Dept. of Histopathology at GOSH. Tel: 020 7829 8663. Fax: 020 7813 1170.

U (urine)
Send 5 ml urine. Keep frozen until dispatched and send by 1st class post. This is used for our metabolic assays, not enzymes. Dilute urines (creatinine <1.0mmol/L) and infected urines (pH >8.0) are unsuitable.

L (liver) M (muscle) J (jejunal)
Contact the enzyme laboratory for instructions before taking liver and muscle biopsies as some assays require the biopsy in an unfrozen state. These assays are only available with prior arrangement and when the tissue sample can be delivered to this laboratory within 1 hour after being taken. Unfrozen samples must be transported in a sealed container on wet ice.

For most enzyme assays, including disaccharidases, a frozen biopsy is required. After wrapping in aluminium foil, the sample must be frozen **immediately,** using solid CO_2 or liquid nitrogen, then placed in a labelled plastic bag. The sample must be stored and transported frozen. **It is essential that the sample remains frozen at all times until it is assayed.**

APPENDIX 2: Perchlorate Precipitation of Samples for Lactate/Pyruvate Ratios and Acetoacetate

For each sample, prepare 2 tubes each with 500 µl of ice cold 0.46 mol/L perchloric acid, keep cold at the bedside on an ice pack. Collect blood into a lithium heparin tube or CSF into a plain tube and IMMEDIATELY pipette 100 µl of the sample into each of the perchloric acid tubes. Mix vigorously, transport to the laboratory on the ice pack. Centrifuge within 10 minutes at 4°C, 3000 rpm for 5 minutes. Freeze supernatant in separate tubes and transport frozen. Any delay in sample precipitation will result in rapid deterioration of the analyte level. Our method requires that the proportion and concentration of perchloric acid is strictly adhered to in order to produce reliable results. Manufacturers supply perchloric acid at a variety of strengths. Please prepare the working perchloric acid as specified below:

NB: For β-hydroxybutyrate / acetoacetate ratio, a separate unprecipitated plasma sample should be sent.

Stock perchloric acid Supplied by manufacturer	Preparation of 0.46 mol/L perchloric acid
60 % w/w (SG 1.54)	2.50 ml stock made up to 50 ml with distilled water
70 % w/w (SG 1.70)	1.94 ml stock made up to 50 ml with distilled water

Keep the working reagent in a plastic bottle at 4 °C.

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